LECTURE 10
BASICS OF RADIATION MICROBIOLOGY FOR FOOD PROTECTION

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INTRODUCTION

Microbes are normally present in unprocessed foods, as well as in many processed foods. Their mere presence in a food generally does not present a health risk, nor does it result in overt spoilage. The risk lies in the capacity of undesirable micro-organisms to multiply in certain foods and cause either food poisoning or food spoilage. The lethal effect of ionizing radiation on micro-organisms, which may either eliminate pathogenic bacteria or reduce the effective rate of multiplication of spoilage bacteria, forms the rationale for ionizing energy treatment of many foods.

In this lecture the microbiological basics of food poisoning, food spoilage, and ionizing energy treatments are presented, factors influencing the microbial resistance of ionizing radiation, including the use of physical agents for combination treatments, are briefly reviewed, and parameters involved in dose selection are considered. Further information may be obtained from the selected references cited.

FOOD POISONING

Food poisoning caused by micro-organisms falls into two main categories.

Food-borne Infections

Food-borne infections from eating contaminated food or drinking contaminated water cause illness by microbial invasion of the host or by release of toxins produced when the food-borne bacteria have grown in the intestinal tract or some other organ. Food-borne infections can be triggered by relatively high numbers of the following genera of bacteria: Salmonella, Shigella, certain strains of Escherichia coli, Vibrio parahaemolyticus, Yersinia enterocolitica, Campylobacter fetus, subsp. jejuni, Clostridium perfringens, Bacillus cereus. Although 10^6 organisms per gram of food are normally required to cause bacterial enteritis symptoms in people, it is well to remember that in certain circumstances the minimum infective dose may be as low as 1 to 10 organisms, as the infectious dose can be affected by the diet, physical condition and immune status of the consumer as well as by other factors (Mossel, 1982).

Food-borne Intoxications

Food borne intoxications cause illness when toxins synthesized in a food by the multiplication and metabolism of certain micro-organisms are absorbed via the intestinal tract of the consumer. Organisms which are responsible for food-borne intoxications are: Clostridium botulinum, Staphylococcus aureus, Bacillus cereus, Pseudomonas cocovenenans, several species of bacteria which metabolize amino acids to form "pressor" amines (histamine, tyramine, and phenylethylamine), and many types of moulds synthesizing mycotoxins. Generally 10^4-10^5 viable cells per gram or ml of food are needed for exotoxins to be released into the food but, as with food-borne infections, other factors may influence the minimum infectious dose.

Infectious Disease Transmission

Other infectious diseases may be transmitted by foods, often after consuming very low numbers of cells only. Examples of micro-organisms, protozoa, and helminths (worms) which may be spread through food are: Corynebacterium

diphtheriae, Group A streptococci, Coxiella burnetii (causes Q fever), Hepatitis A virus and other viruses (e.g. Norwalk), Toxoplasma (Isospora), Entamoeba histolytica, Giardia lamblia, Taenia saginata and T. solium (tapeworms), Trichinella spiralis, Echinococcus (hydatidosis), Anisakis marina (herring worm disease), Capillaria philippinensis (nematode).

Prognosis

Many of the potential pathogenic hazards associated with contaminated food are removed when food is properly processed, properly stored, and properly cooked before consumption. Nevertheless, food poisoning episodes often occur and can be expected with increasing frequency and severity because of changes to intensified animal husbandry practices and large scale production of food to cater for the trend in Western Societies towards increased consumption of meals outside the home.

The number of recorded cases of salmonellosis in the Netherlands increased 13% from 1978 to 1981 (Mossel, 1982). However, it is difficult to obtain accurate figures for the current incidence of food-borne diseases, and under-reporting by 90 to 99% has been suspected (Mossel, 1982). Reported cases of isolation of salmonellae sp. from humans numbered 9000 in the Netherlands and about 25,000 in the USA in 1980. Economic losses in the USA were estimated at US$300,000,000 (Kampelmacher, 1982).

MICROBIAL SPOILAGE OF FOODS

Microbial spoilage occurs in different foods when various organisms develop and cause undesirable biochemical changes in the food. The spoilage pattern is generally characteristic for a specific food and the post-harvest handling conditions and will be dealt with in detail in other lectures. However, in general terms, according to Mossel (1982) the microbial spoilage pattern is the net result of four groups of parameters which limit or permit

proliferation of micro-organisms. These parameters are intrinsic factors of the physical, chemical, and biological properties of the food, food processing modification of the initial microflora, extrinsic factors of the storage environment of the food, implicit factors influencing the organisms selected through the other three parameters. These parameters will be briefly considered.

Intrinsic Factors in Food Spoilage

These are water activity, acidity (pH), oxidation reduction (redox) potential, nutrients, natural inhibitors.

Bacteria need water to multiply in food. If the moisture content of foods is reduced, either by drying or by adding solutes such as salt, sucrose, or a gelling agent to bind the water, and "free" water is unavailable to the bacteria for replication. Water activity, a_w, of a food is the ratio of the water vapour pressure over a food to the vapour pressure of pure water, which is taken as 1.00. There is a a_w limit below which different groups of micro-organisms cannot function normally, although they remain viable (Fig. 1). Thus although bacteria cannot develop when the a_w is less than 0.87, moulds and certain yeasts can continue to proliferate unchecked by multiplication of bacteria.

Most bacteria grow best at near neutral pH whereas yeasts and moulds grow best under acid conditions. The inability of Clostridium botulinum to grow below pH 4.5 means that acid canned foods can be processed at lower temperatures than those normally used.

Spoilage or pathogenic bacteria may proliferate best in the presence of oxygen, or in a limited supply of oxygen, or in the absence of oxygen. The way that foods are stored and packed can therefore affect the type of spoilage organisms that will survive and multiply.

Microbial spoilage also depends on the ability of the invading bacteria to use the chemicals in the foods as substrates. This generally means that the organisms with the appropriate enzymes prefer for example amylolytic, proteolytic, or pectolytic enzymes for spoilage of starches, meats, or fruits respectively. The presence of naturally occurring bacterial inhibitors in foods does not automatically prevent spoilage because generally organisms resistant to the inhibitors are also present.

Influence of Processing in Food Spoilage

Heat treatment is the most common processing method in commercial use, followed by the addition of chemical preservatives such as sulphur dioxide or benzoic or sorbic acids. Ionizing energy treatment will be discussed separately.

Extrinsic Factors in Food Spoilage

Foods stored at chill temperatures will eventually spoil from proliferation of psychrophilic and psychotrophic organisms whereas no microbial growth will occur in foods frozen at about -20°C, rather non-spore-forming organisms will be slowly inactivated at this temperature.

The effect of water vapour pressure during storage on spoilage can be complex and may be influenced by the nature of the food, packaging, moisture content of the food, the a_w gradient, changes in day and night temperatures.

Oxygen depletion and increased partial pressure of carbon dioxide in vacuum packaged foods also affect microbial spoilage. It is important to be aware that anaerobic organisms may grow under aerobic conditions and vice versa.

Implicit Influences on Microbial Spoilage

These may be: different growth rates for organisms of different genera; synergistic growth between groups of micro-organisms caused by increased availability of nutrients or favourable changes in pH, redox value, a_w, elimination of antimicrobial substances, collapse of biological structure; antagonism between microorganisms caused by competitive utilization of nutrients, changes in pH and in redox potential, formation of antibacterial substances, lysis by phages (Table 1).

TABLE 1. ANTAGONISM BETWEEN FOOD PATHOGENS AND SPOILAGE ORGANISMS IN FOODS*

PATHOGEN	ANTAGONIST	
Cl. botulinum	B. subtilis	Brevibact. linens
	Cocci	Cl. sporogenes
	Str. lactis	Enterobacteriacea
	Ps. aeruginosa	Lactobacillaceae
Cl. perfringens	Lactobacillaceae	Cl. sporogenes
	Streptococci D	
Salmonellae	E. coli	Pseudomonas spp
Staph. aureus	Aeromonas	Enterobacteriaceae
	Bacillus sp	Lactobacillaceae
	Streptococci	Staph. epidermidis
	Pseudomonas	Acinetobacter

(Mossel (1982))

MICROBIOLOGICAL BASIS FOR IONIZING ENERGY TREATMENTS OF FOOD

Food preservation methods aim to exert some degree of control over the normal progression of microbial growth in foods for the purpose of delaying or eliminating spoilage or preventing the transmission of food-borne diseases. All these objectives can be achieved with ionizing energy treatment of foods.

Radurization ("radiare" = to radiate, "durare" = to prolong) eliminates some spoilage organisms, thereby reducing total microbial numbers with a consequent extension of the shelf-life of the product. The process is comparable to heat pasteurization.

Radicidation ("radiare" = to radiate, "caedere" = to kill) eliminates all of a specific type of organism which can cause spoilage or food poisoning, thereby reducing the risk of consuming food pathogens.

Radappertization eliminates all organisms from foods or packaging materials to produce a sterile product. The process is comparable to canning.

Biological contaminants of foods vary in resistance to the lethal effects of ionizing energy. Resistance increases inversely with size from parasites, moulds, bacteria (excluding spores), yeast, bacterial spores, with viruses generally being the most resistant. However, within these classes, exceptions and overlapping occurs.

Microbial cells subjected to ionizing energy may continue to respire and move around and may even divide a few times. However, unless they can continue reproducing in a nutrient medium sufficiently to produce colonies, they are regarded as having been inactivated. Loss of colony-forming ability is thus usually taken as the criterion for cell death.

Decimal Reduction Dose (D_{10})

To quantify the sensitivity of organisms to ionizing energy, the number of colony-forming units surviving different doses is used to construct semi-logarithmic plots of the fraction of organisms surviving at different doses (Fig. 2). Similar plots are used to study the resistance of micro-organisms to heat or ethylene oxide or other chemicals.

The radiation resistance of a particular organism tested under specific conditions is determined by the slope of the straight line portion of the graph, as the decimal reduction dose, or D_{10} or D value. The D_{10} dose is the amount of absorbed radiation (dose) needed to reduce the population by 90%, and thus to achieve a 10% survival level of the organisms initially present. It is therefore equivalent to the dose which will reduce the fraction of surviving organisms by one log cycle.

The higher the D_{10} value, the more resistant the organism is to ionizing radiation. However, D_{10} values are not immutable as the resistance of a specific organism can depend on a number of factors. Tables in textbooks which list D_{10} values can therefore be misleading. An alternative method is used in Table 2 where D_{10} values have been used to group food pathogens and spoilage organisms into different radiation sensitivity classes. An organism may belong to more than one class as its D_{10} varies with environmental or other factors.

TABLE 2. COMPARATIVE RADIATION RESISTANCE OF FOOD ORGANISMS

ORGANISM	D_{10} RANGE (kiloGray)			
	0.03-0.25	0.25-0.8	0.8-1.7	1.7-8
	SENSITIVE	MOD.SENS.	MOD.RES.	RESISTANT
Vibrio				
Yersinia				
Campylobacter				
Pseudomonas				
E.coli				
Salmonella				
Staphylococcus				
Penicillium				
Aspergillus				
Micrococcus				
Saccharomyces				
B.coagulans				
B.stearothermophilus				
B.cereus				
Cl.sporogenes				
Cl.perfringens				
Cl.botulinum				
Viruses				

Fortunately, most microbial food pathogens, except for bacterial spores and viruses, are sensitive or moderately sensitive to the effects of ionizing energy. A few food-borne organisms, such as Micrococcus radiodurans and Moraxella/Acinetobacter sp. have been isolated with a very high resistance to ionizing radiation. The survival of these very resistant organisms after moderate doses of ionizing energy is not considered to be a public health hazard.

Effect on Cells

Different food pathogens vary in their resistance to ionizing energy because of inherent differences in:

(1) Amount of water in the cytoplasm
(2) Number of nuclei in the cell
(3) Structure of the chromosomal DNA and its association with repair and degradative enzymes. The base composition of the DNA does not determine radiation resistance. Thus the guanosine-cytosine content of DNA from very resistant M. radiodurans and very sensitive Pseudomonas organisms is the same 67%.
(4) Size of the chromosomal DNA target

In general, the susceptibility of a cell to the adverse effects of ionizing energy can be inversely correlated with the amount of DNA in the cell. The smaller the organism, the greater its resistance. However, differences in radiation sensitivity within a class or species may simply reflect the presence or absence of efficient mechanisms within the cell for repairing radiation-induced damage to the DNA.

Ionizing energy may affect cells either (1) directly or (2) indirectly through free radicals or other radiolysis products formed from liquids surrounding the cell or the vital components within the cell.

The primary "target" for the ionizing energy is most probably the cell's DNA. The association of the DNA with the cytoplasmic membrane is an important secondary target for ionizing radiation. Many DNA repair enzymes are located in the membrane.

The mechanism of radiation damage to the DNA molecule is unclear. Covalent bonds may be broken with a loss of purine or pyrimidine base, leading to a lethal mutation, or the chain may break.

Single strand breaks in the DNA molecule have been measured in bacteria and many other types of cell after exposure to ionizing energy. Breaks in adjoining strands (double strand breaks) generally, but not always, result in cell death.

The fate of single strand breaks varies with environmental conditions and the presence or absence of different enzymes. Strand breaks may be repaired by several different mechanisms, some or all of which also operate when organisms are damaged by heat or chemicals. There is nothing special about the way bacteria respond to the effects of ionizing energy.

FACTORS INFLUENCING RADIATION RESISTANCE OF MICRO-ORGANISMS

The lethal effects of ionizing energy may be influenced by alterations in the environment before, during, and after treatment. Thus different doses of radiation may be required to achieve the same degree of bacterial safety in products prepared or packaged in different ways. Factors which affect radiation resistance include growth phase, gaseous atmosphere, temperature, water activity, dose rate, radiosensitizers, radioprotectants, sub-lethal injury, combination treatments with physical agents. Complex interactions between factors may also occur. A summary of some of the chief effects of these factors on radiation resistance follows.

Growth Phase

Bacteria in the exponential growth phase are generally most sensitive to ionizing energy. Resistance is highest in the lag and stationary phases. However, the reverse situation has been reported for a very resistant organism. Resistance is much greater when bacteria are transformed to dormant spores with part, but not all, of this resistance being lost on germination.

Gaseous Atmosphere

Bacterial sensitivity increases two to five-fold if oxygen is present during treatment. If linear accelerators are used as the source of ionizing energy, atmospheric oxygen within the field is converted to ozone and treatment is therefore substantially anoxic, irrespective of whether or not the product has been vacuum packaged. For dry foods, sensitivity is also influenced by the post-treatment atmosphere. Inactivation is greater when bacterial spores are exposed to oxygen after treatment.

Temperature

The temperature at which a product is treated with ionizing energy may influence the resistance of micro-organisms. Experiments with suspensions of pure cultures of microorganisms at temperatures down to -180°C have shown that the organisms were up to seven or eight times more resistant than when at 25°C. This increase in resistance at low temperature occurs because much of the ability of ionizing energy to harm bacteria indirectly through powerful radiolytic products produced in water is lost with freezing. Inactivation will therefore be limited to that mainly resulting from ionizing energy acting directly on microbial DNA.

Microbial resistance in frozen foods is increased about two or three-fold, compared with resistance at ambient temperature. The D_{10} of Staphylococcus aureus suspended in chicken purée rose from 0.17 kGy at 25°C to 0.57 kGy at -20°C (Fig. 2).

By contrast, bacteria become more sensitive when ionizing treatment is carried out at higher temperatures. This is thought to occur because the repair systems which normally operate at ambient or slightly above ambient temperatures are damaged at higher temperatures.

Water Activity (a_w)

Just as freezing protects bacteria against the lethal effects of ionizing energy, so does a reduction in the moisture content (a_w) of the food. The absorption of ionizing energy in foods of low a_w results in lower concentrations of harmful radiolysis products than in similarly treated high a_w foods.

Bacterial spore resistance appears to be less affected by changes in a_w than vegetative cell resistance. The influence of a_w of different foods and food ingredients on the resistance of specific food pathogens has received little attention.

Dose Rate

For a specific preservation application, the time needed to treat foods could vary from seconds to several hours, depending on whether treatment was undertaken in a facility using a machine or isotopes as the ionizing energy source. It could therefore be important to know whether radiation resistance of bacteria is affected by the rate at which a specific dose is absorbed into a food. This question remains unresolved, with opposing evidence suggesting that electrons are about 10% less efficient than gammas, or the reverse, or that there is no difference between them.

In theory, it is possible that a small irradiator with a very low isotope loading, such that the dose rate was less than 30 Gy h^{-1}, could create a problem for certain foods treated in the summer. If the food is a good culture medium, the multiplication rate of certain organisms

might be higher than the rate at which they can be inactivated. In practice, this situation would be most unlikely to occur because the economics of radiation processing would be too unfavourable.

Radiosensitizers

Many experiments have been carried out to find chemicals which could be added to food to sensitize micro-organisms to the effects of ionizing energy. The theory is that it should then be possible to reduce the dose needed to inactivate the organism and thus help to maintain the organoleptic and essential characteristics of the food. The problem is that food, by its very nature, contains the kind of molecules which may either scavenge free radicals capable of damaging bacteria or else react with the added chemical or its radiolytic products.

The choice of chemicals suitable for testing as sensitizers has generally been based on mechanisms proposed for how the chemicals would act, such as suppression of sulphydryl groups, adverse effects on repair processes, oxygen mimics, abstraction of electrons from ionized biomolecules, production of radiolysis compounds toxic to micro-organisms. In practice, few of the chemicals proposed could be considered suitable additives for foods. However, some success as radiosensitizers has been obtained with sodium chloride and other alkali halides, sodium nitrite and nitrate, and also sorbates.

For fundamental reasons, the degree of sensitization cannot exceed that obtained with oxygen, that is, about a two to four-fold increase in sensitivity. This limitation suggests that combinations of ionizing energy with physical agents could be more effective treatments for promoting radiosensitization.

Radioprotectants

The lethal effect of bacteria in liquid suspension may be reduced in the presence of certain chemicals such as hydrogen sulphide, aliphatic alcohols, glycerol, sucrose, dimethyl sulphoxide, thiourea. These chemicals act by reducing the oxygen content or the water activity level. With the possible exception of sucrose, none of these chemicals could be considered as normal food additives.

Sub-lethal Injury

Sometimes micro-organisms become damaged after treatment with higher doses of ionizing energy. This may also happen to organisms after freezing or heating. The practical effect is that colonies may not be formed under standard plating conditions and special recovery procedures may be needed to allow the injured cells to repair before enumeration takes place. If these are not carried out, the organism will appear to be more sensitive to ionizing energy than it actually is.

Sub-lethal injury may be detected if exponential survival curves began in depart from linearity and become more convex at higher doses, so that it appears that the D_{10} value is decreasing with increasing dose. These types of curves are also seen if inhibitory amounts of food substrate are present in the dilution being plated. They are misleading optical illusions, theoretically impossible, and should always be investigated.

COMBINATION TREATMENTS WITH PHYSICAL AGENTS

The rationale for combining ionizing energy treatment with physical agents to preserve foods is to increase the lethal effectiveness of the radiation, save energy, reduce costs, reduce throughput times, maintain food quality. The physical agents which have been investigated include heat, UV,and hydrostatic pressure. Mild to moderate heat appears the most promising.

Heat

A combination of mild heat and ionizing energy can produce a lethal effect which is synergistic, the effect being greater than the additive effect of the two agents acting independently. The order in which the treatments are supplied influences the result and may depend on the species of organism. Thus inactivation is synergistic when fungal spores are mildly heated and then irradiated. The synergistic effect decreases with increasing delay between treatment. However, for bacterial spores synergism only occurs if the spores are irradiated first and then heated, or if the treatments are simultaneous. Ionizing energy can induce germination-like changes in dormant spores and in this form the spores are more heat sensitive.

A much greater synergistic effect has also been noted when irradiated bacterial spores are heated at 80°-90° and up to 500,000-fold increases in spore inactivation have been reported. It has been suggested that the synergistic effect may be due to lethal heat inactivating repair enzymes.

UV

The possibility of preserving foods with a combination of non-ionizing (UV) and ionizing radiations (gamma or electrons) has been investigated. Further work is needed to clarify the efficiency of this combination.

Hydrostatic Pressure

The dose required to inactivate bacterial spore contaminants of liquids or semi-solid foods may be considerably reduced if applied after moderate hydrostatic pressure treatment carried out at the optimum conditions for maximum (99.9%) inactivation of the particular spore species. Some, but not all, non-spore-forming organisms are also inactivated at similar pressures.

Pre-irradiation compression of sugar solutions experimentally inoculated with B. pumilus spores and the sorbate-resistant yeast Saccharomyces bailii effected about 50% reduction in the dose needed to inactivate 10^6 organisms (Table 3).

TABLE 3. COMBINATION HYDROSTATIC PRESSURE/IONIZING ENERGY TREATMENT FOR DECONTAMINATION OF SUGAR SOLUTIONS

TREATMENT MPa/°C/min	10^6 INACTIVATION DOSE (kGy) B.pumilus	S.bailii
-	18.4	4.5
105/52/8	10	-
276/10/5	-	2.25

CHOICE OF DOSE

The selection of dose will be influenced by:

(1) microbiological objective, for example, pasteurization, decontamination, elimination of a specific pathogen, or elimination of all microbes

(2) number of organisms causing concern per product unit

(3) D_{10}, of the organism(s) causing concern under the prevailing test conditions

(4) degree of assurance needed that the dose will achieve the objective

The effect of batch size on dose needed for sterilization or elimination of specific pathogens is shown in Table 4. In this hypothetical exercise, the objective is to select a dose which will ensure that not more than one organism survives in either 1, 10^4, or 10^6 packets of a product contaminated by a single type of food pathogen at the level of 100 organisms per unit. Each packet therefore contains 100 organisms. The contaminant has a D_{10} value of 3 kilogray when tested in the product. The total number

of contaminants surviving each increment of 3 kGy is shown in Table 4.

TABLE 4. EFFECT OF DOSE ON REDUCTION IN BACTERIAL NUMBERS

DOSE	NUMBER OF ORGANISMS IN BATCH CONTAINING N UNITS (PRE-TREATMENT COLONY-FORMING ORGANISMS PER UNIT = 100; D_{10} = 3kGy)		
	N		
(kGy)	1	10 000	1 000 000
0	100	1 000 000	100 000 000
3	10	100 000	10 000 000
6	1	10 000	1 000 000
9	0.1	1 000	100 000
12		100	10 000
15		10	1 000
18		1	100
21			10
24			1

This table clearly demonstrates the relationship between batch size and the dose needed to eliminate organisms to a specified level per batch. For units of equal contamination levels, to assure that not more than one organism is left in one unit per batch, a dose of 18 kGy would be required if the batch size is 10,000 units or 24 kGy if the batch size is one million units. Thus absence of growth in small samples tested at a particular dose is a quite inadequate and misleading basis for selecting the dose required to achieve the same objective in larger volumes of material.

By contrast, the dose needed to reduce the number of organisms to a constant level per gram or other unit of product is independent of batch size. Thus, in the example given, 6 kilogray would ensure a contamination level of one organism per packet irrespective of whether one or one million packets was treated.

SELECTED REFERENCES

COLLINS-THOMPSON D.L. (1980) Food spoilage and food-borne infection hazards p.12 *In* The Safety of Foods, H.D. Graham (ed.) 2nd ed., Avi, Westport

GAMMASTER (1982) Food Irradiation Now, Proc. Symp Ede, Netherlands, 1981. Martinus Nijhoff/W.Junk, The Hague.

INTERNATIONAL ATOMIC ENERGY AGENCY (1973). Radiation Preservation of Food, Proc. Symp. Bombay, 1972, IAEA, Vienna.

INTERNATIONAL ATOMIC ENERGY AGENCY (1978) Food Preservation by Irradiation, I, II, Proc. Symp. Wageningen, 1977, IAEA, Vienna.

INTERNATIONAL ATOMIC ENERGY AGENCY (1981). Combination Processes in Food Irradiation, Proc. Symp. Colombo, 1980, IAEA, Vienna.

JOSEPHSON E.S., PETERSON, M.S. (eds) (1983). Preservation of Food by Ionizing Radiation, II CRC, Florida.

KAMPELMACHER, E.H. (1982) Public health aspects of food irradiation p.20 *In* Food Irradiation Now, op. cit.

MOSSEL, D.A.A. (1982) Microbiology of foods, University of Utrecht, Utrecht.

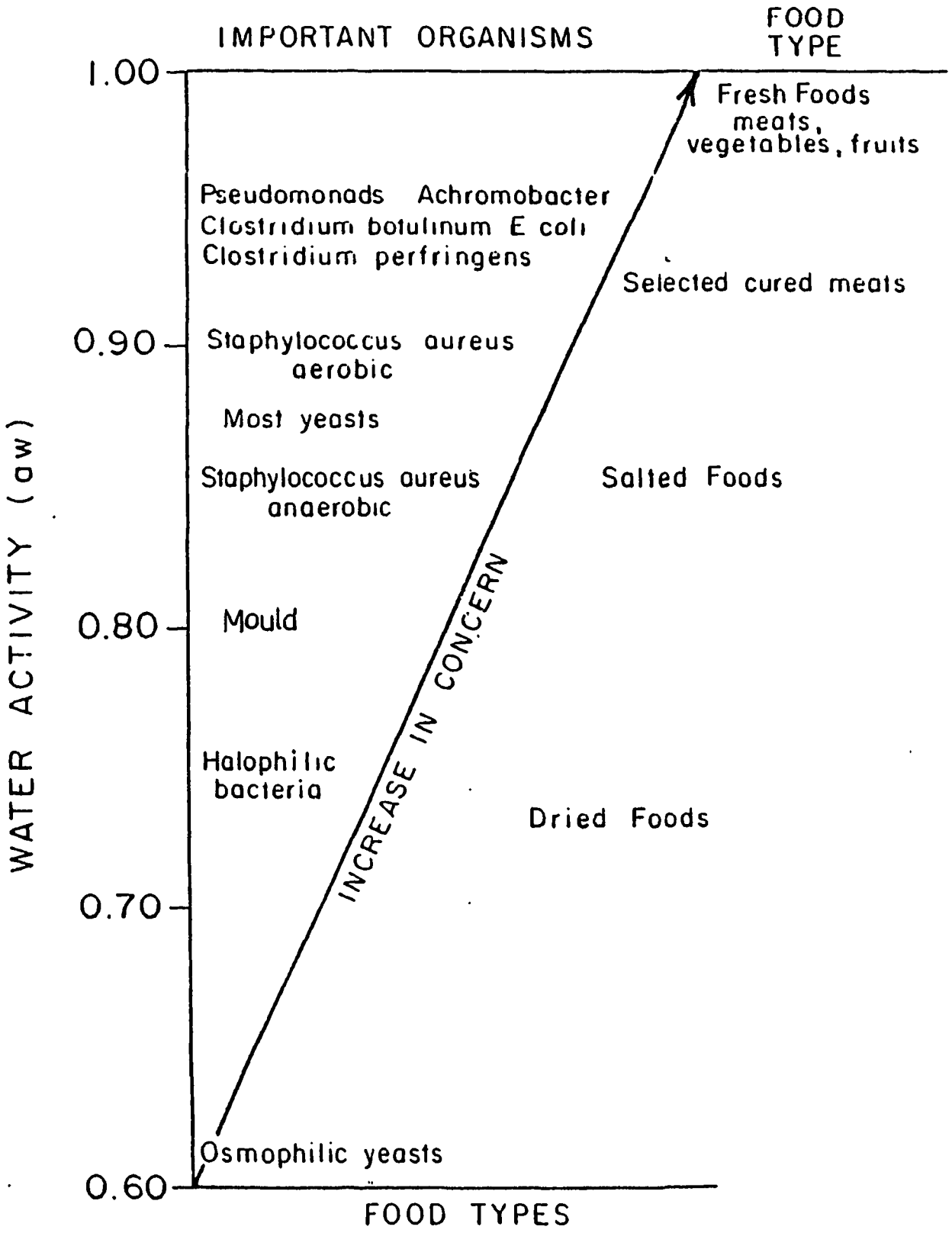

FIG 1. MINIMUM a_w LEVELS OF IMPORTANT ORGANISMS INVOLVED IN SPOILAGE AND FOOD SAFETY

FIG. 2 EFFECTS OF TEMPERATURE ON RADIATION RESISTANCE OF STAPHYLOCOCCUS AUREUS SUSPENDED IN CHICKEN PURÉE.